(12) UK Patent Application (19) GB (11) 2 050 799 A

(21) Application No 8014624
(22) Date of filing 2 May 1980
(30) Priority data
(31) 54/055476
(32) 7 May 1979
(33) Japan (JP)
(43) Application published
14 Jan 1981
(51) INT CL³
A23L 1/34
(52) Domestic classification
A2B 313 323 401 601 603
604 614 615 617 660 BCX
(56) Documents cited
None
(58) Field of search
A2B
(71) Applicants
The Green Cross
Corporation,
1-47, Chuo-1-chome,
Joto-Ku,
Osaka,
Japan.
(72) Inventors
Hiroyuki Okamoto,
Yoshio Tsuda,
Kazumasa Yokayama.
(74) Agents
Mewburn Ellis & Co.

(54) Fat emulsion for intravenous injection and method of preparing it

(57) A stable fat emulsion suitable as a nutritive infusion fluid for intravenous injection contains water and ingredients comprising 5-50 (W/V)% of soybean oil, yolk phospholipids in a weight ratio to the soybean oil of 1/4-1/25, 0.01-0.30 (W/V)% of a fatty acid having 12-20 carbon atoms or a salt thereof, and a cholesterol. The emulsion can be prepared by homogenizing the above ingredients with water, the homogenizing preferably being carried out by admixing the ingredients with water, coarsely emulsifying the resultant admixture and pressure atomizing the coarsely emulsified admixture.

GB 2 050 799 A

SPECIFICATION

Fat emulsion for intravenous injection and method of preparing it

This invention relates to a nutritive infusion fluid and, more particularly, to a fat emulsion for intravenous injection and a method for preparing the same.

In preparing conventional fat emulsion, there have been used emulsifiers such as nonionic surface active agents, yolk phospholipids and soybean phospholipids. The properties of an emulsion naturally vary with the type of emulsifier or of emulsifying aid being used. Since the emulsion, as herein referred to, is a nutritive infusion fluid, it is desirable that the emulsion is used up rapidly in vivo as an energy source after administration. In order that the intravenously injected fat may be rapidly consumed by combustion in the body, it is necessary that the fat does not remain for long periods in blood and that the fat is metabolized without deposition and accumulation in tissues and organs such as the liver and spleen.

For the above reasons, it is necessary to develop an emulsion in which the particles are fine and stable sufficiently. The present inventors found that by the addition of several emulsifiers and emulsifying aids to a conventional fat emulsion for intravenous injection containing soybean oil, water and yolk phospholipids and by subsequent homogenizing it is possible to prepare a fat emulsion which, as compared with known emulsions, has far finer particles, and is far stable and more rapidly utilized as energy source in vivo. Based on this finding, the present invention has been accomplished.

According to this invention, there is provided a fat emulsion for intravenous injection comprising 5 to 50 (W/V)% of soybean oil, yolk phospholipids at a weight ratio to the soybean oil of 1/4 to 1/25, 0.01 to 0.30 (W/V)% of a fatty acid having 12 to 20 carbon atoms or a pharmaceutically acceptable salt thereof, 0.005 to 0.50 (W/V)% of a cholesterol and the balance of water; (W/V)% being the percentage of the weight of a solute or dispersed phase in unit volume of the emulsion.

The soybean oil to be used in preparing the infusion fluid of this invention is a highly purified soybean oil prepared, for example, by the steam distillation method [H.J. Lips, J. Am Oil Chemist. Soc., *27*, 422-423 (1950)] from refined soybean oil, the purity of said highly purified soybean oil being 99.9% or more in terms of the triglyceride, diglyceride and monoglyceride content. Although not subject to particular limitation, the weight ratio of soybean oil to water is generally 0.05 to 0.43, preferably 0.05 to 0.2.

The purified yolk phospholipids used in this invention can be prepared by the usual fractionation with an organic solvent in the following way: To a solution of 130 g of crude yolk phospholipids in a cold mixture of 200 ml of n-hexane and 100 ml of acetone, is added gradually with stirring 1,170 ml of cold acetone. The insolubles are collected by filtration and again dissolved in a cold mixtue of 260 ml of n-hexane and 130 ml of acetone. To the stirred solution, is added 1,170 ml of cold acetone. The insolubles are collected by filtration and freed from the solvent by evaporation to obtain 60 g of a dried substance containing 70 - 80% of phosphatidylcholine, 12 - 25% of phosphatidylethanolamine, and other phospholipids including phosphatidylinositol, phosphatidylserine, sphingomyelin and lyzophosphatidylcholine [D.J. Hanahan et al., J. Biol. Chem., *192*, 623-628 (1951)].

The fatty acids to be used can be those free fatty acids having 12 to 20 carbon atoms which are usable as medicines or pharmaceutically acceptable salts thereof. Examples are stearic acid, oleic acid, linolic acid, palmitic acid, linolenic acid, and potassium or sodium salts thereof. The amount to be used is 0.01 to 0.30, preferably 0.04 to 0.07 (W/V)% as a final concentration in the emulsion. The cholesterols can be those which are suitable for the medical treatment by intravenous injection. The amount to be added is 0.005 to 0.50 (W/V)% as a final concentration in the emulsion.

The emulsion of this invention is prepared in such a manner that a mixture of soybean oil, yolk phospholipid, and a fatty acid having 12 to 20 carbon atoms or a pharmaceutically acceptable salt thereof and a cholesterol is prepared in advance and admixed with water and the resulting mixture is coarsely emulsified by means of a usual mixer, and the coarse emulsion is further homogenized to form the intended emulsion. The homogenizing can be performed in a customary way by means of ultrasonic treatment or pressure atomization. The intended emulsion is obtained, for example, by passing the fluid composition 10 times through a manton-Gaulin homogenizer under application of a pressure of 500 kg/cm^2 [R.P. Geyer et al., J. Am. Oil Chem. Soc., *32*, 365-370 (1955)].

The emulsion of this invention can contain an isotonic agent such as for example glycerol, glucose or the like in an isotonic amount to make the emulsion isotonic. The isotonic agent can be added to the emulsion in the form of an aqueous solution when the coarse emulsion is prepared or thereafter.

As compared with conventional fat emulsions in which soybean phospholipids, nonionic surface active agents, and yolk phospholipids are used as emulsifiers, the fat emulsion for intravenous injection according to this invention is superior in physicochemical stability and in lower side effects. The emulsion of this invention contains dispersed oil droplets having an average diameter of 0.1 μ or less, none of the particles has a diameter exceeding 1 μ, and the state of fine dispersion remains unchanged for a long period of time.

The fat emulsion of this invention showed LD_{50} in rats of 200 ml or more per kg of body weight for a 10% fat emulsion and 150 ml or more for a 20% fat emulsion. No hemolisis is observed on instillation at a normal rate.

Instruction for use of the present preparation: A dose of 300 to 1,000 ml of a 10% of fat emulsion is

administered once a day by intravenous drip. The dose is suitably adjusted in accordance with the body weight and the symptom; and the amount of fat administered intravenously is 2 g (20 ml of the emulsion) or less per day per kg of the body weight.

The invention is illustrated below in detail with reference to Examples and Experimental Examples.

Example 1

To 20.0 g of purified soybean oil, were added 2.4 g of purified yolk phospholipids, 0.05 g of sodium oleate and 0.04 g of cholesterol. The mixture was heated at 65° to 75°C to form a solution. To the solution were added 5.0 g of glycerol and 173 ml of distilled water for injection which had been heated at 65° to 75°C. The resulting mixture was coarsely emulsified by means of a Homomixer. The coarse emulsion was finely emulsified by passing 10 times through a Manton-Gaulin homogenizer under a first stage pressure of 120 kg/cm^2 and a total pressure of 500 kg/cm^2 to obtain a homogenized and very finely dispersed fat emulsion.

Example 2

To 40.0 g of purified soybean oil, were added 2.4 g of purified yolk phospholipids, 0.05 g of sodium oleate and 0.04 g of cholesterol. The mixture was heated at 65° to 75°C to form a solution. To the solution were added 5.0 g of glycerol and 173 ml of distilled water for injection which had been heated at 65° to 75°C. The resulting mixture was coarsely emulsified by means of a Homomixer. The coarse emulsion was finely emulsified by passing 10 times through a Manton-Gaulin homogenizer under a first stage pressure of 120 kg/cm^2 and a total pressure of 500 kg/cm^2 to obtain a uniform and finely dispersed fat emultion.

Experimental Example 1

A comparative experiment was conducted on the stability of emulsion in relation to the composition of emulsifier. Emulsion samples were prepared in a manner similar to that in Example 1, using four emulsifier systems comprising purified yolk phospholipids alone, a combination of purified yolk phospholipids and cholesterol or a free fatty acid, or a combination (according to this invention) of purified yolk phospholipids, cholesterol and a free fatty acid.

The particle size of each emulsion sample was measured by means of an electron microscope immediately after the preparation and after the storage for 24 months at 4°C. The electron microscope used was model JEM-T_s7 of Nippon Denshi Co. The average particle diameter was determined by measuring the particle size from the photograph taken by the carbon replica technique. It was found that the emulsion prepared by using an emulsifier system comprising purified yolk phospholipids, a free fatty acid and cholesterol had uniform and fine particles which remained stable without significant deterioration for a long period of time, indicating that this emulsion is the most excellent of the four emulsions (Table 1).

TABLE 1

Particle diameter of emulsion and stability for storage

Sample No.	*Emulsifier*	*Particle dia. of fat emulsion (μ)*	*Particle dia. after storage for 24 months at 4°C (μ)*
I	*Purified yolk phospholipids*	*0.15 ± 0.03*	*0.25 ± 0.06*
II	*Purified yolk phospholipid and cholesterol*	*0.13 ± 0.03*	*0.20 ± 0.03*
III	*Purified yolk phospholipid and fatty acid*	*0.09 ± 0.02*	*0.13 ± 0.03*
IV	*Purified yolk phospholipids, cholesterol and fatty acid (this invention)*	*0.08 ± 0.02*	*0.11 ± 0.03*

Experimental Example 2

Four fat emulsions were prepared in the same manner as in Experimental Example 1, except that soybean oil having ^{14}C labelled linolic acid in the structure was used. Four groups of Wistar-strain male rats (each 150 g in body weight), which had been fasted for 16 hours, were administered through tail vein with the above emulsions, respectively, at a dose of 20 ml (2 g as soybean oil) per kg of body weight. After the injection, the expiratory air of each rat was collected continually for 6 hours and the radioactivity was measured to compare the emulsions with one another for the metabolic rate of fat as energy source. After the above experiment, each rat was sacrificed and subjected to laparotomy to determine the remaining radioactivity in the plasma, live, spleen and lung.

Further, the emulsions were injected into tail vein of rats in the same manner as mentioned above. Blood

samples were collected from the eyeground of each rat after 10, 15, 20, 30, 60, 90, 120 and 180 minutes from the injection. The neutral fat content of the plasma separated by centrifugation was measured by the acetylacetone method to determine the half-life ($T_{\frac{1}{2}}$) of neutral fat in the plasma. The results obtained were as shown in Table 2.

TABLE 2

Sample No.	Half-life (T½) in plasma (minute)	Recovered radioactivity in expired air during 6 hours after administration (% based on dose)	Distribution of radioactivity after 6 hours from administration			
			Plasma	Liver	Spleen	Lung
I	18.5	20.1 ± 2.5*	0.4 ± 0.05*	46.1 ± 6.4*	5.2 ± 1.0*	2.3 ± 0.1
II	28.3	25.3 ± 2.1	1.3 ± 0.2	31.5 ± 2.8	2.4 ± 0.4	1.8 ± 0.2
III	34.4	27.6 ± 3.1	1.6 ± 0.2	30.1 ± 5.8	1.8 ± 0.3	1.5 ± 0.1
IV	35.2	29.7 ± 2.6	1.5 ± 0.1	33.6 ± 4.9	2.2 ± 0.5	1.8 ± 0.03

Note:
* Significant difference from the values of IV

As described in the foregoing, it is apparent that as compared with the fat emulsion in which purified yolk phospholipids were used as a sole emulsifier, the fat emulsion prepared by using a free fatty acid and cholesterol as emulsifying aids coincidently with yolk phospholipids contains more finely dispersed particles, which remain uniform for a long period of time, and is utilized more rapidly as energy source in vivo, indicating that in this respect the emulsion of this invention is superior to conventional fat emulsions.

CLAIMS

1. A fat emulsion for intravenous injection comprising from 5 to 50 (W/V)% inclusive of soybean oil, yolk phospholipids in a weight ratio to the soybean oil of from 1/4 to 1/25 inclusive, from 0.01 to 0.30 (W/V)% inclusive of a fatty acid having from 12 to 20 carbon atoms inclusive or a pharmaceutically acceptable salt thereof, from 0.005 to 0.50 (W/V)% inclusive of a cholesterol, and water.

2. An emulsion according to claim 1, wherein the average particle diameter of the emulsified particles is 0.1 μ or less.

3. An emulsion according to claim 1, wherein the diameter of the emulsified particles is not greater than 1 μ.

4. An emulsion according to any one of the preceding claims, wherein the amount of the fatty acid or pharmaceutically acceptable salt thereof present in the composition is from 0.04 to 0.07 (W/V)% inclusive.

5. An emulsion according to any one of the preceding claims, wherein the fatty acid is stearic acid, oleic acid, linoleic acid, palmitic acid or linolenic acid.

6. An emulsion according to any one of the preceding claims wherein, which is made isotonic by the addition of glycerol or glucose.

7. A method for preparing a fat emulsion for intravenous injection which comprises homogenizing a mixture of soybean oil, yolk phospholipid, a fatty acid having from 12 to 20 carbon atoms inclusive or a pharmaceutically acceptable salt thereof and a cholesterol in water, the amounts of respective constituents being from 5 to 50 (W/V)% inclusive of the soybean oil, yolk phospholipids in a weight ratio to the soybean oil from 1/4 to 1/25 inclusive, from 0.01 to 0.30 (W/V)%% inclusive of the said fatty acid or pharmaceutically acceptable salt thereof, from 0.005 to 0.50 (W/V)% inclusive of the cholesterol.

8. A method according to Claim 7, wherein the average particle diameter of the emulsified particles is 0.1μ or less.

9. A method according to Claim 7, wherein the diameter of the emulsified particles is not greater than 1μ.

10. A method according to Claim 7, claim 8 or claim 9, wherein the amount of the fatty acid or pharmaceutically acceptable salt thereof is from 0.04 to 0.07 (W/V)% inclusive.

11. A method according to any one of claims 7 to 10, wherein the fatty acid is stearic acid, oleic acid, linoleic acid, palmitic acid or linolenic acid.

12. A method according to any one of claims 7 to 11, which is made isotonic by the addition of glycerol or glucose.

13. A method according to any one of claims 7 to 12, wherein the homogenizing is carried out by admixing the soybean oil, yolk phospholipids, fatty acid or pharmaceutically acceptable salt thereof and cholesterol with water, coarsely emulsifying the resulting admixture and then pressure atomizing the coarsely emulsified admixture.

14. Compositions according to any one of claims 1 to 6, substantially as herein described and exemplified.

15. Methods according to any one of claims 7 to 13, substantially as herein described and exemplified.

16. Compositions whenever prepared by a method according to any one of claims 7 to 13 and 15.

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon Surrey, 1980.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.